

**Amendment and Response**

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Serial No.: 09/772,598

Confirmation No.: 2967

Filed: January 30, 2001

For: CRYSTALLIZATION AND STRUCTURE DETERMINATION OF STAPHYLOCOCCUS AUREUS NAD SYNTHETASE

Please delete the paragraph beginning at page 10, immediately following line 7 (Table 4).

Please insert the following new paragraph at page 10, immediately following line 7 (Table 4).

Molecule #1	LEU 47	GLN 88	GLU 165	GLU 215
	GLY 48	VAL 94	GLY 169	LEU 221
	ILE 49	ILE 111	PHE 170	ASP 223
	SER 50	PHE 132	TYR 171	ALA 226
	GLY 51	GLY 135	THR 172	TYR 231
	GLY 52	ASN 136	LYS 173	ILE 256
	GLN 53	ALA 139	TYR 174	ARG 257
	ASP 54	ARG 140	GLY 175	ASN 258
	SER 55	ARG 142	ASP 176	ALA 259
	THR 56	GLN 146	GLY 177	HIS 260
	VAL 81	VAL 158	LYS 189	LYS 261
	LYS 82	GLY 159	THR 211	ALA 265
	LEU 83	THR 160	ALA 212	TYR 266
	PRO 84	ASP 161	ASP 213	TRP 271
	TYR 85	HIS 162	LEU 214	
Molecule #2	TYR 1035	GLN 1146	SER 1151	ALA 1179
	HIS 1039	PHE 1147	GLY 1155	ASP 1180
	PHE 1041	SER 1148	ILE 1156	ILE 1181
	ILE 1042	ALA 1150	VAL 1157	

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Please delete the paragraph beginning at page 11, immediately following line 2 (Table 5).

Please insert the following new paragraph at page 11, immediately following line 2 (Table 5).

Molecule #1	VAL 46	VAL 94	THR 160	ASP 217
	LEU 47	GLU 95	ASP 161	LYS 218
	GLY 48	ALA 97	HIS 162	LEU 221
	ILE 49	LEU 98	ALA 164	PRO 222
	SER 50	ILE 101	GLU 165	ASP 223
	GLY 51	VAL 107	ASN 166	GLU 224
	GLY 52	THR 108	THR 168	ASP 225
	GLN 53	VAL 109	GLY 169	ALA 226
	ASP 54	ASN 110	PHE 170	LEU 227
	SER 55	ILE 111	TYR 171	TYR 231
	THR 56	THR 130	THR 172	HIS 254
	LEU 57	ASP 131	LYS 173	TYR 255
	VAL 58	PHE 132	TYR 174	ILE 256
	GLY 59	GLN 133	GLY 175	ARG 257
	PHE 78	LYS 134	ASP 176	ASN 258
	ILE 79	GLY 135	GLY 177	ALA 259
	ALA 80	ASN 136	ALA 178	HIS 260
	VAL 81	GLU 137	ALA 179	LYS 261
	LYS 82	LYS 138	ILE 184	ARG 262
	LEU 83	ALA 139	LYS 189	GLU 263
	PRO 84	ARG 140	LYS 208	LEU 264

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	TYR 85	GLU 141	THR 209	ALA 265
	GLY 86	ARG 142	PRO 210	TYR 266
	VAL 87	MET 143	THR 211	THR 267
	GLN 88	VAL 145	ALA 212	THR 270
	LYS 89	GLN 146	ASP 213	TRP 271
	ASP 90	VAL 157	LEU 214	PRO 272
	ALA 91	VAL 158	GLU 215	
	GLU 93	GLY 159	ASP 216	
Molecule #2	PHE 1031	LEU 1045	ALA 1150	GLY 1159
	TYR 1035	VAL 1046	SER 1151	ALA 1178
	VAL 1036	MET 1143	ASN 1152	ALA 1179
	SER 1038	LYS 1144	ARG 1153	ASP 1180
	HIS 1039	VAL 1145	GLN 1154	ILE 1181
	SER 1040	GLN 1146	GLY 1155	ALA 1182
	PHE 1041	PHE 1147	ILE 1156	
	ILE 1042	SER 1148	VAL 1157	
	SER 1044	ILE 1149	VAL 1158	

Please replace the paragraph beginning at page 25, line 22, with the following rewritten paragraph. Pursuant to 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

B1 The structure coordinates set forth in Table 1 can be used to aid in obtaining structural information about another crystallized molecule or molecular complex. A "molecular complex" means a protein in covalent or non-covalent association with a chemical entity or

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compound. The method of the invention allows determination of at least a portion of the three-dimensional structure of molecules or molecular complexes which contain one or more structural features that are similar to structural features of *S. aureus* NadE. These molecules are referred to herein as "structurally homologous" to *S. aureus* NadE. Similar structural features can include, for example, regions of amino acid identity, conserved active site or binding site motifs, and similarly arranged secondary structural elements (e.g.,  $\alpha$  helices and  $\beta$  sheets). Optionally, structural homology is determined by aligning the residues of the two amino acid sequences to optimize the number of identical amino acids along the lengths of their sequences; gaps in either or both sequences are permitted in making the alignment in order to optimize the number of identical amino acids, although the amino acids in each sequence must nonetheless remain in their proper order. Preferably, two amino acid sequences are compared using the Blastp program, version 2.0.9, of the BLAST 2 search algorithm, as described by Tatusova et al., FEMS Microbiol Lett 174, 247-50 (1999), and available from the world wide web at [ncbi.nlm.nih.gov/gorf/bl2.html](http://ncbi.nlm.nih.gov/gorf/bl2.html). Preferably, the default values for all BLAST 2 search parameters are used, including matrix = BLOSUM62; open gap penalty = 11, extension gap penalty = 1, gap x\_dropoff = 50, expect = 10, wordsize = 3, and filter on. In the comparison of two amino acid sequences using the BLAST search algorithm, structural similarity is referred to as "identity." Preferably, a structurally homologous molecule is a protein that has an amino acid sequence sharing at least 65% identity with the amino acid sequence of *S. aureus* NadE (SEQ ID NO: 1). More preferably, a protein that is structurally homologous to *S. aureus* NadE includes at least one contiguous stretch of at least 50 amino acids that shares at least 80% amino acid sequence identity with the analogous portion of *S. aureus* NadE. Methods for generating structural information about the structurally homologous molecule or molecular complex are well-known and include, for example, molecular replacement techniques.

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and

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Please replace the paragraph beginning at page 44, line 24, with the following rewritten paragraph. Pursuant to 37 C.F.R. §1.121, this paragraph is also shown in Appendix A with notations to indicate the changes made.

B2 The resolution and quality of the anomalous and dispersive signals were poor making data interpretation difficult. A second data set collected on a selenomethionine incorporated NadE crystal grown in the presence of N<sup>a</sup>AD was also very mosaic suggesting a fundamental problem with the selenomethionine crystals and/or the cryogenic conditions used to preserve the crystals. However, the data was of sufficient quality at 2.7Å to begin to identify heavy atom positions. Both anomalous and dispersive difference Patterson maps revealed the presence of at least four strong heavy atom peaks (Figures 2-3). A total of eight sites were identified using automated Patterson interpretation methods in SHELX (Sheldrick et al., Acta Cryst., B51:423-31 (1995)). There are four methionines in each molecule of NadE excluding the N-terminal methionine. Therefore, the eight heavy atom positions would be consistent with the presence of two molecules in the asymmetric unit. Phasing with these eight sites led to electron density maps that were difficult to interpret suggesting that the positions of the heavy atom sites might be incorrect except for the fact that these sites were completely consistent with the Patterson maps. Methionine positions from the initial molecular replacement solutions described above using the NadE dimer from *Bacillus subtilis* were also consistent with the peaks in the Patterson maps making the difficulty in refinement and the low quality electron maps even more puzzling.